



National Environmental
Laboratory **Accreditation**
Conference

QUALITY SYSTEMS

PROPOSED CHANGES

November 18, 2002

NOTE: Additions (double-underlined) and ~~deletions~~ (struck through) to the approved standards being proposed for vote at the next Annual Meeting are marked as in this note.

5.0 QUALITY SYSTEMS

INTRODUCTION

~~Quality Systems include all quality assurance (QA) policies and quality control (QC) procedures, which shall be delineated in a Quality Manual and followed to ensure and document the quality of the analytical data. Laboratories seeking accreditation under NELAP must assure implementation of all QA policies and the essential applicable QC procedures specified in this Chapter. The QA policies, which establish essential QC procedures, are applicable to environmental laboratories regardless of size and complexity.~~

Each laboratory shall have a quality system. The laboratory's Quality System is the process by which the laboratory conducts its activities so as to provide the client with data of known and documented quality with which to demonstrate regulatory compliance and for other decision-making purposes. This system includes a process by which appropriate analytical methods are **selected**, their capability is **evaluated**, and their performance is **documented**. The quality system shall be documented in the laboratory's quality manual.

This chapter contains detailed quality system requirements for consistent and uniform implementation by both the laboratories conducting testing under these standards and the evaluation of those laboratories by accrediting authorities. Each laboratory seeking accreditation under NELAP must assure that they are implementing their quality manual and that all the Quality Control (QC) procedures specified in this Chapter are being followed. The Quality Assurance (QA) policies, which establish essential QC procedures, are applicable to environmental laboratories regardless of size and complexity.

~~The intent of this Chapter is to provide sufficient detail concerning quality system requirements so that all accrediting authorities evaluate laboratories consistently and uniformly.~~

~~NELAC is committed to the use of Performance-based Measurement Systems (PBMS) in environmental testing and provides the foundation for PBMS implementation in these standards. While this standard may not currently satisfy all the anticipated needs of PBMS, NELAC will address future needs within the context of State statutory and regulatory requirements and the finalized EPA implementation plans for PBMS.~~

5.4 MANAGEMENT REQUIREMENTS

5.4.1 Organization

5.4.1.5 The laboratory shall:

- h) have technical management which has overall responsibility for the technical operations and the provision of the resources needed to ensure the required quality of laboratory operations;

The technical director(s) (however named) shall certify that personnel with appropriate educational and/or technical background perform all tests for which the laboratory is accredited. Such certification shall be documented.

The technical director(s) shall meet the requirements specified in the Accreditation Process. (see 4.1.1.1)

- i) appoint a member of staff as quality manager (however named) who, irrespective of other duties and responsibilities, shall have defined responsibility and authority for ensuring that the quality system is implemented and followed at all times; the quality manager shall have direct access to the highest level of management at which decisions are made on laboratory policy or resources;

~~Where staffing is limited, the~~ The quality manager may also be the technical director or deputy technical director when the laboratory has three or less full time employees;

5.4.13 Internal Audits

5.4.13.1 The laboratory shall periodically, in accordance with a predetermined schedule and procedure, and at least annually, conduct internal audits of its activities to verify that its operations continue to comply with the requirements of the quality system and this Standard. The internal audit program shall address all elements of the quality system, including the environmental testing ~~and/or calibration~~ activities. It is the responsibility of the quality manager to plan and organize audits as required by the schedule and requested by management. Such audits shall be carried out by trained and qualified personnel who are, ~~wherever resources permit~~ independent of the activity to be audited. Personnel shall not audit their own activities except when it can be demonstrated that an effective audit will be carried out.

5.4.13.2 When audit findings cast doubt on the effectiveness of the operations or on the correctness or validity of the laboratory's environmental test ~~or calibration~~ results, the laboratory shall take ~~timely~~ corrective action within ten days of the audit report, and shall notify clients in writing if investigations show that the laboratory results may have been affected.

5.5 TECHNICAL REQUIREMENTS

5.5.2 Personnel

5.5.2.6 The laboratory management shall be responsible for:

- c) ensuring that the training of each member of the technical staff is kept up-to-date (on-going) by the following:
 - 3) Analyst training shall be considered up to date if an employee training file contains a certification that technical personnel have read, understood and agreed to perform the most recent version of the test method (the approved method or standard operating procedure as defined by the laboratory document control system, 5.4.2.3.d) and documentation of continued proficiency by at least one of the following once per year:
 - i. acceptable performance of a blind sample (single blind to the analyst); Note: Successful analysis of a blind PT sample used for a similar test method using the same technology [e.g., GC/MS volatiles by purge and trap for Methods 524.2, 624 or 5035/8260] would require documentation for one of the test methods
 - ii. ~~another demonstration of capability; another demonstration of capability~~ an initial measurement system evaluation as defined in Appendix C;
 - iii. ~~successful analysis of a blind performance sample on a similar test method using the same technology (e.g., GC/MS volatiles by purge and trap for Methods 524.2, 624 or 5035/8260) would only require documentation for one of the test methods; The laboratory must determine the acceptable range of the blind performance sample prior to analysis;~~
 - iv. at least four consecutive laboratory control samples with acceptable levels of precision and ~~accuracy~~ bias; or

5.5.4 ~~Environmental Test and Calibration Methods and Method Validation~~

~~5.5.4.1 General~~

~~The laboratory shall use appropriate methods and procedures for all environmental tests and/or calibrations within its scope. These include sampling, handling, transport, storage and preparation of samples, and, where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of environmental test and/or calibration data.~~

~~The laboratory shall have instructions on the use and operation of all relevant equipment, and on the handling and preparation of samples where the absence of such instructions could jeopardize the results of environmental tests and/or calibrations. All instructions, standards, manuals and reference data relevant to the work of the laboratory shall be kept up to date and shall be made~~

~~readily available to personnel (see 5.4.3). Deviation from environmental test and calibration methods shall occur only if the deviation has been documented, technically justified, authorized, and accepted by the client.~~

5.5.4.1 Standard Operating Procedures and Laboratory Manual

The laboratory shall maintain a methods manual consisting, at a minimum, of all the laboratory's standard operating procedures (SOP's). The SOP's shall accurately reflect all phases of current laboratory activities such as sample receipt, sample storage, sample analysis, assessment of data integrity, corrective actions, handling of customer complaints, test methods, and data and record storage. All confidential business information in the methods manual shall be so designated and appropriately marked by the laboratory.

5.5.4.1.1 Standard Operating Procedures (SOPs)

~~Laboratories shall maintain SOPs that accurately reflect all phases of current laboratory activities such as assessing data integrity, corrective actions, handling customer complaints, and all test methods.~~

- a) ~~These documents, for example, may be equipment manuals provided by the manufacturer, or internally written documents. An SOP may be an equipment manual provided by a manufacturer, or an internally written document so long as the SOP is adequately detailed to permit someone other than the analyst to reproduce the procedures that had been used to produce a given result.~~
- b) ~~The test methods may be copies of published methods as long as any changes or selected options in the methods are documented and included in the methods manual (see 5.5.4.1.2). The test method SOP's may be copies of published methods as long as any changes or selected options in the methods are documented and included in the SOP's (see below). Standardized test methods that contain sufficient and concise information on how to perform the tests do not need to be supplemented or rewritten as internal procedures if these methods are written in a way that they can be used as published by the laboratory. It may be necessary to provide additional documentation for optional steps in the method or additional details.~~
- c) ~~Copies of all SOPs shall be accessible to all personnel and organized in a way to be understood by all staff.~~
- d) ~~The SOPs shall be organized.~~
- e) ~~Each SOP shall clearly indicate the effective date of the document, the revision number and the signature(s) of the approving authority.~~
- f) Each test method shall give or reference the following information where relevant (the order in which these items appear in the SOP is left to the discretion of the laboratory staff:
 - 1) Scope and Application
 - 2) Summary of Method
 - 3) Definitions
 - 4) Interferences
 - 5) Safety
 - 6) Equipment and Supplies
 - 7) Reagents and Standards
 - 8) Sample Collection, Preservation, and Storage
 - 9) Quality Control
 - 10) Calibration and Standardization
 - 11) Procedure
 - 12) Data Analysis and Calculations
 - 13) Method Performance

- 14) Pollution Prevention
- 15) Waste Management
- 16) References
- 17) Tables, Diagrams, Flowcharts, and Validation Data

5.5.4.1.2 Laboratory Method Manual(s)

- a) ~~The laboratory shall have and maintain an in-house methods manual(s) for each accredited analyte or test method.~~
- b) ~~This manual may consist of copies of published or referenced test methods or SOPs that have been written by the laboratory. In cases where modifications to the published method have been made by the laboratory or where the referenced test method is ambiguous or provides insufficient detail, these changes or clarifications shall be clearly described. Each test method shall include or reference where applicable.~~
 - 1) ~~identification of the test method;~~
 - 2) ~~applicable matrix or matrices;~~
 - 3) ~~detection limit;~~
 - 4) ~~scope and application, including components to be analyzed;~~
 - 5) ~~summary of the test method;~~
 - 6) ~~definitions;~~
 - 7) ~~interferences;~~
 - 8) ~~safety;~~
 - 9) ~~equipment and supplies;~~
 - 10) ~~reagents and standards;~~
 - 11) ~~sample collection, preservation, shipment and storage;~~
 - 12) ~~quality control;~~
 - 13) **calibration and standardization;**
 - 14) ~~procedure;~~
 - 15) ~~calculations;~~
 - 16) ~~method performance;~~
 - 17) ~~pollution prevention;~~
 - 18) ~~data assessment and acceptance criteria for quality control measures;~~
 - 19) ~~corrective actions for out-of-control data;~~
 - 20) ~~contingencies for handling out-of-control or unacceptable data;~~
 - 21) ~~waste management;~~
 - 22) ~~references; and,~~
 - 23) ~~any tables, diagrams, flowcharts and validation data.~~

5.5.4.2 Selection of Methods

~~The laboratory shall use methods for environmental testing and/or calibration, including methods for sampling, which meet the needs of the client and which are appropriate for the environmental tests and/or calibrations it undertakes.~~

The laboratory shall utilize methods within its scope (including sample collection, sample handling, transport and storage, sample preparation and sample analysis) that are appropriate and applicable to client needs (i.e., to meet regulatory or other requirements specified by the client). These requirements may specify that a particular method or group of methods be employed for a given project or program, or that specific measurement quality objectives be achieved, or both.

When the use of a particular test method is mandated by regulation or requested by a client, only that method shall be used. Deviations from a test method shall occur only if the deviation has been documented, technically justified, authorized, and approved for use by the client. The laboratory shall inform the client when the method proposed by the client is considered not capable of providing data consistent with intended use. Client approval of the methods to be used when conducting analyses must be obtained prior to implementation. Modifications must be documented in reports to the client and/or in any communication with the client.

When the use of a particular test method is neither mandated by regulation nor requested by a client, the laboratory shall select methods that are appropriate for the intended use. Such methods may be those published in international, regional, or national standards, or by reputable technical organizations, or in relevant scientific texts or journals, or as specified by the manufacturer of the equipment, or laboratory-developed methods or methods adapted by the laboratory. The laboratory shall document in reports to its clients all methods utilized in the performance of work.

----- Sections 5.5.4.2.1 - 5.5.4.5.3 struck in their entirety -----

5.5.4.3 Measurement System Evaluation and Performance Demonstration

All measurements made while operating as a NELAC accredited laboratory must have an adequate demonstration that the measurement system provided data consistent with its intended use. This demonstration consists of three activities:

- 1) an initial evaluation that the measurement system is capable of providing data of the quality needed to meet client and/or regulatory requirements (see section 5.5.4.3.1 below and Appendix C);
- 2) an acceptable instrument calibration and, where applicable, a verification that the system has remained calibrated during the period that it was used for analysis (see Section 5.5.5.2.2);
- 3) an on-going demonstration of measurement system performance that documents the laboratory is operating with its analytical system in control as well as a documentation of the quality of data obtained on the actual samples analyzed (see section 5.5.4.3.2 below and Appendix D).

The specific elements required for these activities are summarized in Table 5-1.

<u>Table 5-1. Summary of Critical Elements for the Initial Evaluation and On-going Demonstration of Measurement System Performance</u>		
<u>Evaluation Element</u>	<u>Initial Evaluation of Measurement System Performance</u>	<u>Ongoing Demonstration of Measurement System Performance</u>
<u>Calibration</u>	<u>Calibrate instrument</u>	<u>Calibrate instrument and/or verify calibration</u>
<u>Limit of Detection (LOD)</u>	<u>Establish LOD on each sample-type; if analytes are to be reported at LOD, analyze LOD QC sample. An LOD QC sample is an extracted spike at 2-3 X the determined LOD for single analyte tests, 1-4 X the LOD for multiple analyte tests, and must always be less than the LOQ.</u>	<u>If analytes are to be quantitatively reported at LOD, analyze a LOD QC sample on each instrument once per quarter (or whenever samples are analyzed if less than once per quarter). An LOD QC sample is an extracted spike at 2-3 X the determined LOD for single analyte tests, 1-4 X the LOD for multiple analyte tests, and must always be less than the LOQ.</u>
<u>Limit of Quantitation (LOQ)</u>	<u>Establish LOQ on each sample-type; verify by analysis of LOQ QC sample. Determine acceptance limits for LOQ QC samples</u>	<u>Analyze LOQ QC sample on each instrument annually.</u>
<u>Bias*</u>	<u>Establish acceptance limits for bias on each sample-type</u>	<u>Matrix spike/matrix spike duplicates per method or client requirements**</u>
<u>Precision*</u>	<u>Establish acceptance limits for precision on each sample-type</u>	<u>Matrix spike/matrix spike duplicates or replicate samples per method or client requirements.</u>

<u>Method Range*</u>	<u>Establish working range</u>	<u>No requirement</u>
<u>Selectivity</u>	<u>Establish measures for ensuring selectivity criteria for each SOP</u>	<u>Confirm analyte identity and quantitative reliability for each positive result</u>
<u>Analytical System Performance</u>	<u>No requirement</u>	<u>Analyze Laboratory Control Sample with each batch</u>
<u>System Cleanliness</u>	<u>Analyze Method blank</u>	<u>Analyze Method blank with each batch</u>
<u>Analyst Proficiency</u>	<u>Initial demonstration of proficiency; see Appendix G</u>	<u>Periodic demonstration of proficiency; see Appendix G</u>
<u>Laboratory proficiency</u>	<u>Successful analysis of 2 PT samples; see Chapter 2</u>	<u>Annual analysis of 2 PT samples; see Chapter 2</u>

* The specific requirements vary for standardized and non-standardized methods

** These QC samples are not required when the laboratory clients do not provide samples and/or MQOs.

5.5.4.3.1 Initial Evaluation of Measurement System Performance

Each laboratory must evaluate the capability of its measurement system relative to its intended purpose. Properties of the measurement system to be evaluated include the range, bias, precision, sensitivity, and selectivity. The measurement system includes the analyst (operator) or work cell and test method.

Procedures for the initial measurement system evaluation are presented in Appendix C. When changes are made in a test method, the influence of such changes shall be documented and, if appropriate, a new evaluation shall be carried out.

5.5.4.3.2 On-going Demonstration of Measurement System Performance

In addition to the requirement for an initial evaluation, the following general quality control (QC) procedures, used to demonstrate that the laboratory analytical system was functioning correctly and to document the performance of the test method when used to analyze samples, shall apply. The manner in which they are implemented is dependent on the types of tests performed by the laboratory (i.e., chemical, whole effluent toxicity, microbiological, radiological, air) and are further described in Appendix D. The standards for any given test type shall assure that the applicable principles are addressed:

- a) The laboratory shall have QC procedures in place to demonstrate the performance of the measurement system on an on-going basis, including:
 - 1) procedures to verify that the instrument is calibrated;
 - 2) procedures to ensure that the measurement system is free of laboratory induced interferences;
 - 3) procedures to identify if and when the laboratory is in an out-of-control condition;
 - 4) procedures to document the sensitivity, precision and bias of the results for the samples analyzed;
 - 5) procedures to confirm analyte identity and quantitative accuracy; and
 - 6) procedures to verify analyst proficiency.
- b) All quality control measures shall be assessed and evaluated on an on-going basis, using pre-established quality control (QC) acceptance criteria. (See Appendix D.)
- c) The laboratory shall have procedures for the development of QC acceptance criteria and associated corrective action procedures for all QC activities where no analogous method or regulatory criteria or procedures exist.

- d) The quality control protocols specified by the laboratory's method manual (5.5.4.1.2) shall be followed. The laboratory shall ensure that the essential standards outlined in Appendix D or mandated methods or regulations (whichever are more stringent) are incorporated into their method manuals. When it is not apparent which is more stringent the QC in the mandated method or regulations is to be followed.
- e) To the extent possible, samples shall be reported only if all quality control measures are acceptable. If a quality control measure is found to be out of control and the data is to be reported, all samples associated with the failed quality control measure shall be reported with defined qualifiers or flags or explained in the case narrative.

5.5.4.46 ——— Non-Standard Methods Estimation of Uncertainty of Measurement

~~5.5.4.6.1~~ A calibration laboratory, or an environmental testing laboratory performing its own calibrations and issuing a calibration certificate, shall have and shall apply a procedure to estimate the uncertainty of measurement for all calibrations and types of calibrations.

~~5.5.4.4.12~~ Environmental testing laboratories shall have and shall apply procedures for estimating uncertainty of analytical measurement. In certain cases the nature of the test method may preclude rigorous, metrologically and statistically valid, calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data.

In those cases where a well-recognized test method specifies limits to the values of the major sources of uncertainty of measurement and specifies the form of presentation of calculated results, the laboratory is considered to have satisfied this clause by following the test method and reporting instructions (see 5.5.10).

~~5.5.4.4.25-5.4.6.32~~ When estimating the uncertainty of measurement, all uncertainty components which are of importance in the given situation shall be taken into account using appropriate methods of analysis.

5.5.4.55.5.4.5- Control of Data

~~5.5.4.5.15-5.4.7.1~~ Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Calculations and data transfers shall be subject to appropriate checks in a systematic manner.

- a) The laboratory shall establish SOPs to ensure that the reported data are free from transcription and calculation errors.
- b) The laboratory shall establish SOPs to ensure that all quality control measures are reviewed, and evaluated before data are reported.
- c) The laboratory shall establish SOPs addressing manual calculations including manual integrations.

~~5.5.4.5.25-5.4.7.2~~ When computers, automated equipment, or microprocessors are used for the acquisition, processing, recording, reporting, storage or retrieval of environmental test ~~or calibration~~ data, the laboratory shall ensure that:

- a) computer software developed by the user is documented in sufficient detail and is suitably validated as being adequate for use;
- b) procedures are established and implemented for protecting the data; such procedures shall include, but not be limited to, integrity and confidentiality of data

entry or collection, data storage, data transmission and data processing;

- c) computers and automated equipment are maintained to ensure proper functioning and are provided with the environmental and operating conditions necessary to maintain the integrity of environmental test and calibration and calibration data.
- d) it establishes and implements appropriate procedures for the maintenance of security of data including the prevention of unauthorized access to, and the unauthorized amendment of, computer records.

Commercial off-the-shelf software (e. g. word processing, database and statistical programs) in general use within their designed application range is considered to be sufficiently validated. However, laboratory software configuration or modifications must be validated as in 5.5.4.7.5.2a.

5.5.4.6 Estimation of Uncertainty of Measurement (This section moved to 5.5.4.4)

5.5.4.7 Control of Data (This section moved to 5.5.4.5)

5.5.5 Equipment

5.5.5.2.2 Instrument Calibration

~~This standard specifies the essential elements that shall define the procedures and documentation for initial instrument calibration and continuing instrument calibration verification to ensure that the data must be of known quality and be appropriate for a given regulation or decision. This standard defines the requirements that laboratories must follow to ensure that all instruments used for analysis are properly calibrated before and during their use in order for the data to be of known quality and appropriate for the intended use. This standard does not specify detailed procedural steps ("how to") for calibration, but establishes the essential elements for~~
~~Note: In the following sections, initial instrument calibration is directly used for quantitation and continuing instrument calibration verification is used to confirm the continued validity of the initial calibration unless otherwise required by regulation, method, or program.~~

5.5.5.2.2.1 Initial Instrument Calibration

The following items are essential elements of initial instrument calibration:

- d) All initial instrument calibrations must be verified with a standard obtained from a second manufacturer or lot if the lot can be demonstrated from the manufacturer as prepared independently from other lots. Traceability shall be to a national standard, when commercially available.
- f) The lower calibration standard shall be at or below the limit of quantitation and the upper calibration standard at the highest concentration that quantitative data are to be reported (see Appendix C). These two calibration standards define the working range of the calibration.
- g) Measured concentrations that are outside of the working range shall be reported as having less certainty (e.g., defined qualifiers or flags or explained in the case narrative). The lowest demonstrated limit of quantitation is the lowest concentration that data shall be reported with certainty.
- h) g)If the initial instrument calibration results are outside established acceptance criteria, corrective actions must be performed and all associated samples reanalyzed. If reanalysis of the samples is not possible, data associated with an unacceptable initial instrument calibration shall be reported with appropriate data qualifiers.
- h) ~~Calibration standards must include concentrations at or below the regulatory limit/decision level, if these limits/levels are known by the laboratory, unless these concentrations are below the laboratory's demonstrated detection limits (See D.1.4~~

~~Detection Limits)~~

5.5.5.5 Records shall be maintained of each major item of equipment and its software significant to the environmental tests ~~and/or calibrations~~ performed. The records shall include at least the following:

- d) the current location, ~~where appropriate;~~

5.5.5.10 ~~When intermediate checks are needed to maintain confidence in the calibration status of the equipment, these checks shall be carried out according to a defined procedure. When an initial instrument calibration is not performed on the day of analysis, the validity of the initial calibration shall be verified prior to sample analyses by a continuing instrument calibration verification with each analytical batch. Calibration shall be verified before conducting any analyses and at the end of each analytical batch.~~ The following items are essential elements of continuing instrument calibration verification:

- a) The details of the continuing instrument calibration procedure, calculations and associated statistics must be included or referenced in the test method SOP.
- b) ~~A continuing instrument calibration verification must be repeated at the beginning and end of each analytical batch. The concentrations of the calibration verification shall be varied within the established calibration range. If an internal standard is used, only one continuing instrument calibration verification must be analyzed per analytical batch. Calibration shall be verified for each compound, element, or other discrete chemical species, except for mixtures such as Aroclor-1254, Total Petroleum Hydrocarbons, or Toxaphene where a representative chemical related substance or mixture can be used.~~
- c) Instrument calibration verification must be performed:
- (1) at the beginning and end of each analytical batch (however, if an internal standard is used, only one verification needs be performed at the beginning of the analytical batch).
 - (2) whenever it is suspected that the analytical system may be out of calibration or might not meet the verification acceptance criteria.
 - (3) if the time period for calibration or the most previous calibration verification has expired, or
 - (4) for analytical systems that contain a calibration verification requirement based on the number of runs and the number of runs is exceeded.
- d) Sufficient raw data records must be retained to permit reconstruction of the continuing instrument calibration verification, e.g., test method, instrument, analysis date, each analyte name, concentration and response, calibration curve or response factor, or unique equations or coefficients used to convert instrument responses into concentrations. Continuing calibration verification records must explicitly connect the continuing verification data to the initial instrument calibration.
- e) Criteria for the acceptance of a continuing instrument calibration verification must be established, e.g., relative percent difference.
- f) If the continuing instrument calibration verification results obtained are outside established acceptance criteria, corrective actions must be performed. If routine corrective action procedures fail to produce a second consecutive (immediate) calibration verification within acceptance criteria, then either the laboratory has to demonstrate acceptable performance after corrective action with two consecutive ~~successful~~ calibration verifications, or a new initial instrument calibration must be performed. ~~If the laboratory has not demonstrated acceptable performance, sample analyses shall not occur until a new initial calibration curve is established and verified. However, sample data associated with an unacceptable calibration verification may be reported as qualified~~

~~data under the following special conditions. If the laboratory has not verified calibration, sample analyses should not occur until the analytical system is calibrated or calibration is verified. If samples are analyzed using a system on which the calibration has not been verified the results shall be flagged accordingly, and the reasons discussed in the narrative. Data associated with an unacceptable calibration verification may be fully useable under the following special conditions:~~

5.5.6.2 Specific Requirements Testing Laboratories

----- Sections 5.5.6.2.1 - 5.5.6.2.2 struck in their entirety -----

~~**5.5.6.2.2.1 Calibration Laboratories** For testing laboratories, the requirements given in 5.5.6.2.1 apply for measuring and test equipment with measuring functions used, unless it has been established that the associated contribution from the calibration contributes little to the total uncertainty of the test result. Then this situation arises the laboratory shall ensure that the equipment used can provide the uncertainty of measurement needed.~~

- ~~a) The overall program of calibration and/or verification and validation of equipment shall be designed and operated so as to ensure that measurements made by the laboratory are traceable to national standards of measurement.~~
- ~~b) Calibration certificates shall indicate the traceability to national standards of measurement and shall provide the measurement results and associated uncertainty of measurement and/or a statement of compliance with an identified metrological specification. The laboratory shall maintain records of all such certifications.~~

~~**5.5.6.2.2.2** Where traceability of measurements to SI units is not possible and/or not relevant, the same requirements for traceability to, for example, certified reference materials, agreed methods and/or consensus standards, are required as for calibration laboratories (see 5.5.6.2.1.2). The laboratory shall provide satisfactory evidence of correlation of results, for example by participation in a suitable program of interlaboratory comparisons, proficiency testing, or independent analysis.~~

- ~~a) The overall program of calibration and/or verification and validation of equipment shall be designed and operated so as to ensure that measurements made by the laboratory are traceable to national standards of measurement.~~
- ~~b) Calibration certificates shall indicate the traceability to national standards of measurement and shall provide the measurement results and associated uncertainty of measurement and/or a statement of compliance with an identified metrological specification. The laboratory shall maintain records of all such certifications.~~
- ~~c) Where traceability to national standards of measurement is not applicable, the laboratory shall provide satisfactory evidence of correlation of results, for example by participation in a suitable program of interlaboratory comparisons, proficiency testing, or independent analysis.~~

5.5.6.3 Reference Standards and Reference Materials

5.5.6.3.1 Reference Standards

The laboratory shall have a program and procedure for the calibration of its reference standards. Reference standards shall be calibrated by a body that can provide traceability as described in 5.5.6.2.4.4. Such reference standards of measurement held by the laboratory (such as class S or equivalent weights or traceable thermometers) shall be used for calibration only and for no other purpose, unless it can be shown that their performance as reference standards would not be invalidated. Reference standards shall be calibrated before and after any adjustment. Where possible commercially available, this traceability shall be to a national standard of measurement.

5.5.6.3.2 Reference Materials

Reference materials shall, where ~~possible commercially available~~, be traceable to SI units of measurement, or to certified reference materials. Where ~~possible commercially available~~, traceability shall be to national or international standards of measurement, or to national or international standard reference materials. Internal reference materials shall be checked as far as is technically and economically practicable.

5.5.8.3.1 Sample Receipt Protocols

- a) All items specified in 5.5.8.3.2 below shall be checked.
 - 1) All samples which require thermal preservation shall be considered acceptable if the arrival temperature is either within 2°C of the required temperature or the method specified range. For samples with a specified temperature of 4°C, samples with a temperature ranging from just above the freezing temperature of water to 6°C shall be acceptable. Samples that are hand delivered to the laboratory ~~immediately~~ within one hour after collection may not meet ~~this~~ these criteria. In these cases, the samples shall be considered acceptable if there is evidence that the chilling process has begun such as arrival on ice.

5.5.10.3 Supplemental Information for Test Reports

- b) where ~~relevant quality system requirements are not met~~, a statement of compliance/non-compliance with requirements and/or specifications, including identification of test results derived from any sample that did not meet NELAC sample acceptance requirements such as improper container, holding time, or temperature;

----- Sections 5.5.10.4 - 5.5.10.8 struck in their entirety -----

5.5.11 Documentation

The documentation of the results from the Initial Evaluation of measurement system performance (see Table C-1), the instrument calibration (see section 5.5.5.2.2) and Ongoing Demonstration of measurement system performance (see Table D-1) shall be maintained with the laboratory records and reported to the client along with the actual test results when appropriate or requested. In addition, client-specified measurement quality objectives and laboratory-derived measurement quality characteristics shall be documented

Appendix B - CERTIFICATION OF ANALYST PROFICIENCYDate:Laboratory Name:Laboratory Address:Analyst Name:Sample Type:SOP Number:Parameters:We, the undersigned, CERTIFY that:

- n) The analyst identified above, using the cited laboratory Standard Operating procedure (SOP), has demonstrated proficiency to conduct the analyses.
- o) The demonstration was performed according to the procedures of Chapter 5 (5.5.2.6(c)4) of the National Environmental Laboratory Accreditation Conference standards.
- p) The data associated with this demonstration are true, accurate, complete and self-explanatory.
- q) All raw data necessary to reconstruct and validate this certification have been retained by the facility and are available for review by authorized parties.

Technical Director's Name Signature _____ Date _____Quality Assurance Officer's Name Signature _____ Date _____Analyst's Name Signature _____ Date _____

Date: _____ Page _____

___ of ___

Laboratory Name: _____

Laboratory Address: _____

Analyst(s) Name(s): _____

Matrix: _____

(examples: laboratory pure water, soil, air, solid, biological tissue)

Method number, SOP#, Rev#, and Analyte, or Class of Analytes or Measured Parameters

(examples: barium by 200.7, trace metals by 6010, benzene by 8021, etc.)

We, the undersigned, CERTIFY that:

_____ 1. The analysts identified above, using the cited test method(s), which is in use at this facility for the analyses of samples under the National Environmental Laboratory Accreditation Program, have met the Demonstration of Capability.

_____ 2. The test method(s) was performed by the analyst(s) identified on this certification.

_____ 3. A copy of the test method(s) and the laboratory-specific SOPs are available for all personnel on-site.

_____ 4. The data associated with the demonstration capability are true, accurate, complete and self-explanatory (1).

_____ 5. All raw data (including a copy of this certification form) necessary to reconstruct and validate these analyses have been retained at the facility, and that the associated information is well organized and available for review by authorized assessors.

Technical Director's Name and Title _____ Signature _____ Date _____

Quality Assurance Officer's Name _____ Signature _____ Date _____

This certification form must be completed each time a demonstration of capability study is completed.

_____ (1) True: Consistent with supporting data.

Accurate: Based on good laboratory practices consistent with sound scientific principles/practices.

Complete: Includes the results of all supporting performance testing.

Self-Explanatory: Data properly labeled and stored so that the results are clear and require no additional explanation

Appendix C - INITIAL EVALUATION OF MEASUREMENT SYSTEM PERFORMANCE

----- Sections C.1 struck in its entirety -----

----- Section C.2 (Certification Statement) moved to Appendix B -----

C.1 Purpose

This Appendix serves to assess whether or not a particular measurement system is suitable for an intended purpose. This appendix also defines the documentation necessary to provide evidence that a measurement system was appropriately evaluated. The laboratory shall ensure that the essential requirements in this Appendix are incorporated into their method manuals and/or the Laboratory Quality Assurance Plan.

The activities specified in this appendix are not suitable for demonstrating that a method, when considered independent of a laboratory's quality system, is valid. That activity generally requires a collaborative study such as is described in ASTM D-2777.

C.2 Background

The basis for evaluating whether a measurement system's performance is suitable for a particular purpose are Measurement Quality Objectives (MQOs). The MQO elements are sensitivity, range, precision, bias, and selectivity. The measurement system is a test method as implemented at a particular laboratory (i.e., the laboratory SOP, equipment and staff).

The range, bias, precision and sensitivity characteristics of each measurement system are determined by the laboratory using the procedures in this Appendix. These measures of system performance are defined as the measurement quality characteristics (MQCs). MQCs may be periodically updated as more data on the performance of the test method is generated. The selectivity of the measurement system is evaluated as part of the bias evaluation. If MQCs do not meet the respective MQOs, then the measurement system does not yield data suitable for its intended purpose.

An initial evaluation is done when the laboratory implements a test method for the first time, significantly modifies a test method that has previously been evaluated by the laboratory, adds an analyte to an existing method, or uses an existing test method for a different sample-type. This evaluation is performed to demonstrate that the laboratory and test method (the measurement system) are capable of providing data of the quality needed and to ensure data suitable for the intended purpose. The activities required for this evaluation are summarized in Table C-1.

When laboratory clients or regulation provide the laboratory with the required MQOs, these MQOs shall be used. When MQOs are not provided to the laboratory, the laboratory may use the performance characteristics of published standardized test methods as MQOs, or may establish MQOs based on the MQC data. If the measurement system performance is not adequate (i.e., does not meet one or more of the MQO requirements) for the intended purpose, the laboratory must notify the client prior to the analysis of client samples. If the test method is modified to achieve improved MQOs, the initial evaluation must be repeated.

The role of the laboratory includes:

- Evaluate client MQOs and specific method requirements, if any, to assist in the method selection process.
- Determine if the MQOs can be met and provide assurance of said performance to the client.
- Update MQCs from ongoing measurement system demonstration (See Appendix D) as appropriate.
- Employ test methods that meet the needs of the client and ensure data suitable for its intended use.

C.3 Initial Evaluation – General

The initial evaluation must be performed for all methods used at the laboratory, including:

- standardized test methods with no modifications;
- standardized test methods that have been modified, and
- laboratory developed test methods.

This evaluation (Section C.5) demonstrates the laboratory's ability to use the test method correctly. Sample specific modifications (i.e., using a smaller sample size; adding a cleanup step) can be performed without re-doing the evaluation as long as the following conditions are met:

- the changes can be scientifically justified as not being ones that would change the nature of the procedure;
- the appropriate ongoing quality control sample analyses are used to document the measurement system performance; and
- both the changes and the rationale for the changes not having to be evaluated using the initial evaluation procedure are documented (e.g., SOP, case narrative, corrective action form, non-conformance memo).

When a new analyte is added to an existing test method, an initial evaluation must be performed for that analyte. Section C.5 details the steps involved in the initial evaluation. The bias and precision evaluation in Section C.5.2.2 must be followed for new analytes, unless the laboratory can demonstrate, by the nature of the analyte being added, that all measures of system performance can be assured (e.g., isomer of previously evaluated constituent that does not exhibit chromatographic interference with other target analytes) In the latter case, the bias and precision steps in Section C.5.2.1 must be followed. In other words, the initial evaluation must be sufficient to support the intended use of the data.

C.4 Matrices, Sample-Types and Quality Control Samples

An initial evaluation must be performed for every test method in the laboratory and for every sample-type to which the test method is applied. The sample-types described below refer to a sample with certain properties within the broadly defined NELAC matrices, (Drinking Water; Non-Potable Water; Solid and Chemical Materials; Biological Tissues; and Air and Emissions) that provides a reasonable challenge to the test method, but that does not address all potential matrix issues that could exist in actual samples. If the test method is to be used on sample matrices which provide a more significant analytical challenge (e.g., sludges, chemical wastes, oils, brines), the on-going demonstration activities must be used to document the performance of the test method in these other matrices. Alternatively, the laboratory may perform an initial evaluation on these sample matrices. Laboratories also have the option to perform the initial evaluation on samples collected from a specific site (e.g., a POTW can use the wastewater discharged from their facility) provided the test method is only used to analyze those samples or samples with comparable characteristics.

The sample-type for the Drinking Water matrix is tap water from the laboratory.

The Non-Potable Water sample type (based on ASTM D-5905) shall be prepared as follows:

500 mL water
0.4 g flour
2 g ocean salts
0.08 g Kaolin
120 mL beer

Dilute to 2 L

If the initial evaluation is performed on a Non-Potable Water sample-type, then the method may be applied to all Drinking Water and Non-Potable Water samples. However, if the initial evaluation is performed on a Drinking Water sample-type, the method may be applied to other sample-types within the Drinking Water matrix only.

For the Solid and Chemical Materials matrix the appropriate sample-type is a soil or sediment containing at least 10% each of sand, silt and clay and at least 5% moisture.

Within the Biological Tissues matrix the appropriate sample type is any fish or animal tissue that contains at least 5% fat.

Within the Air and Emissions matrix separate initial evaluations are required for canister or other whole-volume air samples, Polyurethane foam plugs (PUF) samples, filter media or the various absorption tube media.

The evaluation requires the laboratory to analyze various quality control (QC) samples. These samples may be:

- Certified Reference Materials (CRMs),
- The sample-types described above, fortified by spiking, or
- Actual samples, where the concentration of the analyte is known, either by an analysis using a different technology or based on spiking.

C.5 Measurement System Evaluation Elements

The table below summarizes each of the elements that must be evaluated and the requirements for performing the evaluation.

<u>Table C-1. Summary of Initial Measurement System Evaluation Elements and Requirements*</u>	
<u>Evaluation Element:</u>	<u>Evaluation Requirements</u>
<u>Limit of Detection</u>	<p><u>Determine LOD by EPA (40 CFR 136, App. B) or other established procedure for each analyte for each sample-type; include qualitative analyte identification and isolation/concentration steps, as appropriate. LOD determinations based on variance, such as the EPA MDL procedure, should be designed to reflect the variance inherent in normal lab operations. For example, the replicates should be spread over as long a time period as possible. Other options may be available, depending on agency/program requirements.</u></p> <p><u>If analytes are to be quantitatively reported at LOD, analyze a LOD QC sample on each instrument. A LOD QC sample is an extracted spike at 2-3 X the determined LOD for single analyte tests, 1-4 X the LOD for multiple analyte tests, and must always be less than the LOQ. The acceptance criterion for the LOD sample is detection of all analytes. If any analytes are not detected then the LOD is increased and the test repeated.</u></p>
<u>Limit of Quantitation</u>	<p><u>Determine LOQ.</u></p> <p><u>Verification of calculated LOQ done by analysis of a QC sample containing analytes at LOQ. See Section C.5.1.2.</u></p>
<u>Range**</u>	<p><u>Establish the working range for each analyte as part of the calibration procedure. The working range is defined by the high and low calibration standards, or as defined by the method.</u></p>
<u>Precision & Bias: standardized methods**</u>	<p><u>Analyze QC sample containing analytes at 2-5X LOQ prior to any isolation/concentration steps; perform in quadruplicate. Verify & document reliable qualitative identification of analytes. Calculate % recovery and RSD for each analyte. Perform prior to implementation or when measurement system changes significantly.</u></p>
<u>Precision & Bias: non-standardized methods**</u>	<p><u>Analyze QC sample in triplicate at three concentrations, the LOQ, mid-range, and upper-range. Analyze a method blank with each replicate set. Use a CRM for the mid-range QC sample if available. Process as three sets of samples through the entire measurement system for each analyte of interest. Each set, covering the concentration range of interest, should be processed and assayed</u></p>

	<u>on separate days. Verify & document reliable qualitative identification of analytes. For each analyte, calculate the mean % recovery for each day, for each level over days, and for all nine samples. Calculate the relative std. deviation for each of the means obtained. Perform prior to implementation or when measurement system changes significantly.</u>
<u>Selectivity</u>	<u>Incorporate appropriate tests for selectivity in the method. The evaluation for selectivity is done as part of the evaluation of bias.</u>

* Each of these elements must be evaluated prior to using a test method and when the measurement system changes significantly.

**The range, precision and bias of the measurement system do not need to be evaluated if the objective of the analysis is to demonstrate absence/presence of the analyte at the LOQ.

C.5.1 Evaluation of Sensitivity

C.6 Evaluation of Sensitivity

C.5.1.1 Limit of Detection

The Limit of Detection (LOD) shall be established for every analyte in each sample-type for which data are to be reported. If a regulation specifies a specific technique for determining the LOD, it shall be followed. In other instances any procedure for establishing the LOD that exists in EPA regulations or guidance or in the peer-reviewed literature, may be used. LOD determinations based on variance should be performed on each instrument in use, and, depending on the procedure used, may also include a measure of variance over time. For reporting purposes, instrument specific LODs may be used, or, alternatively, the highest LOD for each given analyte determined for a given test on similar instruments may be used. When clients request quantitative data be reported to the LOD, then the validity of the detection limit determination must be demonstrated by qualitative identification of the analyte in a QC sample containing the analyte at no more than 2-3X the LOD for single analyte tests and 1-4X the LOD for multiple analyte tests. This verification must be performed on every instrument that is to be used for analysis of samples and reporting of data.

The LOD must be determined each time there is a change in the test method that affects how the test is performed, or when a change in instrumentation occurs that affects the sensitivity of the analysis.

All sample processing steps of the test method shall be included in the determination of the LOD. Instrument detection limits (determinations made without all sample processing steps required by the method) are not acceptable substitutes for the above referenced determinations.

All procedures used must be documented. Documentation must include the sample-type. All supporting data must be retained.

An LOD study is not required for any component or property for which spiking solutions or quality control samples are not available, or otherwise inappropriate (e.g., pH).

C.5.1.2 Limit of Quantitation

The minimum limit of quantitation (LOQ) shall be established for every analyte for which quantitative data are to be reported. The minimum level of quantitation is the lowest value for which unqualified quantitative data may be reported by the laboratory. When determining the LOQ, if client, regulatory agency, or other requirements are in place, those requirements shall be followed. In other instances, any procedure for establishing the LOQ may be used as long as the validity of the determination is confirmed by successful analysis of a QC sample containing the analytes of concern at or near the claimed LOQ. A successful analysis is one where the recovery of each analyte is within the established MQOs. This single analysis is not required if the bias and precision of the measurement system is evaluated at the LOQ as described in Section 5.2.2.

The level of the lowest calibration standard shall be approximately equivalent to the LOQ.

If project-specific MQOs have quantitation limit requirements greater than the LOQ, the laboratory may, alternatively, analyze a QC sample containing the analyte at the lowest concentration of concern. All sample processing steps of the analytical protocol shall be included in the determination of the LOQ.

An LOQ study is not required for any component or property for which spiking solutions or quality control samples are not available or otherwise inappropriate (e.g., pH).

C.5.2 Evaluation of Bias and Precision

If sample results are to be reported over a concentration range, the bias and precision of the method must be evaluated over the working range. If the objective of the sample analyses is to only demonstrate the presence or absence of an analyte at a specific concentration, or to establish whether or not the concentration is above or below a specified value, then this determination need not be completed.

C.5.2.1 Standardized Methods

The following approach can be used for standardized methods, i.e., test methods published by an organization generally recognized as competent to do so. The approach in section C.5.2.2 below is required for modifications to standardized methods, laboratory-developed methods, or methods published in the scientific literature.

For each method and sample-type, the laboratory must analyze four replicate QC samples containing each analyte at 2-5 times the LOQ, or as otherwise stated in the method. The samples must be processed through all sample preparation and analysis steps in the method. The mean recovery and relative standard deviation are used to establish the laboratory-derived MQCs. The MQCs are compared to the MQOs required by the client or regulation. If MQOs are not provided by the client or required by regulation, the laboratory-derived MQCs become the MQOs.

C.5.2.2 Non-standardized Methods

This approach can be used for any method but is required for modifications to standardized methods, laboratory-developed methods, or methods published in the scientific literature. This approach is also required if a client provides MQOs that are different from those published in standardized methods. This approach may be used by the laboratory to document the performance of the method over the concentration range of interest for standardized methods if the laboratory chooses to perform this study.

Analyze QC samples in triplicate containing the analyte at or near the quantitation limit, at the upper-range of the calibration (upper 20%) and at a mid-range concentration. If a Certified Reference Material (CRM) of the same matrix type as the samples is available, substitute the CRM for the appropriate replicates. Process these samples as three sets of samples through the entire measurement system for each analyte of interest. Each day one QC sample at each concentration is analyzed. A separate method blank shall be subjected to the analytical method along with the QC samples on each of the three days. (Note that the three samples at the LOQ concentration demonstrate sensitivity as well.) For each analyte, calculate the mean recovery for each day, for each level over days, and for all nine samples. Calculate the relative standard deviation for each of the separate means obtained.

Compare the results at each concentration to see if there is a significant difference in either bias or relative precision as a function of concentration. If there is no significant difference, calculate the mean recovery and relative standard deviation over the range of interest by combining all values. If there is a significant difference, calculate the mean recovery and relative standard deviation at each concentration. Evaluate the blank data for an indication of a positive bias. Compare these calculated results to the MQOs and determine if the method is adequate for its intended use. If no MQOs exist, the laboratory shall use these data to establish the MQCs.

C.5.3 Evaluation of Selectivity

The minimum requirement is to ensure that the measurement system is adequately selective. Appropriate selectivity checks established within the method should be followed, including mass spectral tuning, second column confirmation, ICP inter-element interference checks, chromatography retention time windows and related activities.

Appendix D - ESSENTIAL QUALITY CONTROL REQUIREMENTS

The quality control protocols specified by the laboratory's method manual (5.5.4.1.2) shall be followed. The laboratory shall ensure that the essential standards outlined in Appendix D are incorporated into their method manuals and/or the Laboratory Quality Manual.

All quality control measures shall be assessed and evaluated on an on-going basis and quality control acceptance criteria shall be used to determine the validity of the data. The laboratory shall have procedures for the development of acceptance/rejection criteria where no method or regulatory criteria exists.

The requirements from the body of Chapter 5, e.g., 5.5.9.2, apply to all types of testing. The specific manner in which they are implemented is detailed in each of the sections of this Appendix, i.e., chemical testing, W.E.T. testing, microbiology testing, radiochemical testing and air testing.

~~D.1 CHEMICAL TESTING~~

~~D.1.1 Positive and Negative Controls~~

~~a) Negative Control - Method Performance~~

~~Purpose: The method blank is used to assess the preparation batch for possible contamination during the preparation and processing steps. The method blank shall be processed along with and under the same conditions as the associated samples to include all steps of the analytical procedure. Procedures shall be in place to determine if a method blank is contaminated. Any affected samples associated with a contaminated method blank shall be reprocessed for analysis or the results reported with appropriate data qualifying codes.~~

~~Frequency: The method blank shall be analyzed at a minimum of 1 per preparation batch. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.~~

~~Composition: The method blank shall consist of a matrix that is similar to the associated samples and is known to be free of the analytes of interest.~~

~~Evaluation Criteria and Corrective Action: While the goal is to have no detectable contaminants, each method blank must be critically evaluated as to the nature of the interference and the effect on the analysis of each sample within the batch. The source of contamination shall be investigated and measures taken to minimize or eliminate the problem and affected samples reprocessed or data shall be appropriately qualified if:~~

~~1. The concentration of a targeted analyte in the blank is at or above the reporting limit as established by the test method or by regulation, AND is greater than 1/10 of the amount measured in any sample.~~

~~2. The blank contamination otherwise affects the sample results as per the test method requirements or the individual project data quality objectives.~~

~~b) Positive Control—Method Performance~~~~1) Laboratory Control Sample (LCS)~~

~~Purpose: The LCS is used to evaluate the performance of the total analytical system, including all preparation and analysis steps. Results of the LCS are compared to established criteria and, if found to be outside of these criteria, indicates that the analytical system is "out of control". Any affected samples associated with an out of control LCS shall be reprocessed for re-analysis or the results reported with appropriate data qualifying codes.~~

~~Frequency: The LCS shall be analyzed at a minimum of 1 per preparation batch. Exceptions would be for those analytes for which no spiking solutions are available such as total suspended solids, total dissolved solids, total volatile solids, total solids, pH, color, odor, temperature, dissolved oxygen or turbidity. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.~~

~~Composition: The LCS is a controlled matrix, known to be free of analytes of interest, spiked with known and verified concentrations of analytes. NOTE: the matrix spike may be used in place of this control as long as the acceptance criteria are as stringent as for the LCS. Alternatively the LCS may consist of a media containing known and verified concentrations of analytes or as Certified Reference Material (CRM). All analyte concentrations shall be within the calibration range of the methods. The following shall be used in choosing components for the spike mixtures:~~

~~The components to be spiked shall be as specified by the mandated test method or other regulatory requirement or as requested by the client. In the absence of specified spiking components the laboratory shall spike per the following:~~

~~For those components that interfere with an accurate assessment such as spiking simultaneously with technical chlordane, toxaphene and PCBs, the spike should be chosen that represents the chemistries and elution patterns of the components to be reported.~~

~~For those test methods that have extremely long lists of analytes, a representative number may be chosen. The analytes selected should be representative of all analytes reported. The following criteria shall be used for determining the minimum number of analytes to be spiked. However, the laboratory shall insure that all targeted components are included in the spike mixture over a 2-year period.~~

~~a) — For methods that include 1-10 targets, spike all components;~~

~~b) — For methods that include 11-20 targets, spike at least 10 or 80%, whichever is greater;~~

~~c) — For methods with more than 20 targets, spike at least 16 components.~~

Evaluation Criteria and Corrective Action: ~~The results of the individual batch LCS are calculated in percent recovery or other appropriate statistical technique that allows comparison to established acceptance criteria. The laboratory shall document the calculation.~~

~~The individual LCS is compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory shall determine internal criteria and document the method used to establish the limits or utilize client specified assessment criteria.~~

~~A LCS that is determined to be within the criteria effectively establishes that the analytical system is in control and validates system performance for the samples in the associated batch. Samples analyzed along with a LCS determined to be "out of control" shall be considered suspect and the samples reprocessed and re-analyzed or the data reported with appropriate data qualifying codes.~~

c) ~~Sample Specific Controls~~

~~The laboratory must document procedures for determining the effect of the sample matrix on method performance. These procedures relate to the analyses of matrix specific Quality Control (QC) samples and are designed as data quality indicators for a specific sample using the designated test method. These controls alone are not used to judge laboratory performance.~~

~~Examples of matrix specific QC include: Matrix Spike (MS); Matrix Spike Duplicate (MSD); sample duplicates; and surrogate spikes. The laboratory shall have procedures in place for tracking, managing, and handling matrix specific QC criteria including spiking appropriate components at appropriate concentrations, calculating recoveries and relative percent difference, evaluating and reporting results based on performance of the QC samples.~~

~~Matrix Spike; Matrix Spike Duplicates:~~

Purpose: ~~Matrix specific QC samples indicate the effect of the sample matrix on the precision and accuracy of the results generated using the selected method. The information from these controls is sample/matrix specific and would not normally be used to determine the validity of the entire batch.~~

Frequency: ~~The frequency of the analysis of matrix specific samples shall be determined as part of a systematic planning process (e.g. Data Quality Objectives) or as specified by the required mandated test method.~~

~~Composition: The components to be spiked shall be as specified by the mandated test method. Any permit specified analytes, as specified by regulation or client requested analytes shall also be included. If there are no specified components, the laboratory shall spike per the following:~~

~~For those components that interfere with an accurate assessment such as spiking simultaneously with technical chlordane, toxaphene and PCBs, the spike should be chosen that represents the chemistries and elution patterns of the components to be reported.~~

~~For those test methods that have extremely long lists of analytes, a representative number may be chosen using the following criteria for choosing the number of analytes to be spiked. However, the laboratory shall insure that all targeted components are included in the spike mixture over a 2 year period.~~

- ~~a) For methods that include 1-10 targets, spike all components;~~
- ~~b) For methods that include 11-20 targets, spike at least 10 or 80%, whichever is greater;~~
- ~~c) For methods with more than 20 targets, spike at least 16 components.~~

~~Evaluation Criteria and Corrective Action: The results from matrix spike/matrix spike duplicate are primarily designed to assess the precision and accuracy of analytical results in a given matrix and are expressed as percent recovery (%R), relative percent difference (RPD), or other appropriate statistical technique that allows comparison to established acceptance criteria. The laboratory shall document the calculation for %R, RPD or other statistical treatment used.~~

~~The results are compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory shall determine internal criteria and document the method used to establish the limits. For matrix spike results outside established criteria corrective action shall be documented or the data reported with appropriate data qualifying codes.~~

d) ~~Matrix Duplicates:~~

~~Purpose: Matrix duplicates are defined as replicate aliquots of the same sample taken through the entire analytical procedure. The results from this analysis indicate the precision of the results for the specific sample using the selected method. The matrix duplicate provides a usable measure of precision only when target analytes are found in the sample chosen for duplication.~~

~~Frequency: The frequency of the analysis of matrix duplicates may be determined as part of a systematic planning process (e.g. Data Quality Objectives) or as specified by the mandated test method.~~

~~Composition: Matrix duplicates are performed on replicate aliquots of actual samples. The composition is usually not known.~~

~~Evaluation Criteria and Corrective Action:~~ The results from matrix duplicates are primarily designed to assess the precision of analytical results in a given matrix and are expressed as relative percent difference (RPD) or another statistical treatment (e.g., absolute differences). The laboratory shall document the calculation for relative percent difference or other statistical treatments.

~~Results are compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory shall determine internal criteria and document the method used to establish the limits. For matrix duplicates results outside established criteria corrective action shall be documented or the data reported with appropriate data qualifying codes.~~

~~e) Surrogate Spikes:~~

~~Purpose:~~ Surrogates are used most often in organic chromatography test methods and are chosen to reflect the chemistries of the targeted components of the method. Added prior to sample preparation/extraction, they provide a measure of recovery for every sample matrix.

~~Frequency:~~ Except where the matrix precludes its use or when not available, surrogate compounds must be added to all samples, standards, and blanks for all appropriate test methods.

~~Composition:~~ Surrogate compounds are chosen to represent the various chemistries of the target analytes in the method. They are often specified by the mandated method and are deliberately chosen for their being unlikely to occur as an environmental contaminant. Often this is accomplished by using deuterated analogs of select compounds.

~~Evaluation Criteria and Corrective Action:~~ The results are compared to the acceptance criteria as published in the mandated test method. Where there are no established criteria, the laboratory should determine internal criteria and document the method used to establish the limits. Surrogates outside the acceptance criteria must be evaluated for the effect indicated for the individual sample results. The appropriate corrective action may be guided by the data quality objectives or other site specific requirements. Results reported from analyses with surrogate recoveries outside the acceptance criteria should include appropriate data qualifiers.

~~D.1.2 Detection Limits~~

~~The laboratory shall utilize a test method that provides a detection limit that is appropriate and relevant for the intended use of the data. Detection limits shall be determined by the protocol in the mandated test method or applicable regulation, e.g., Method Detection Limit (MDL). If the protocol for determining detection limits is not specified, the selection of the procedure must reflect instrument limitations and the intended application of the test method.~~

~~a) A detection limit study is not required for any component for which spiking solutions or quality control samples are not available such as temperature.~~

~~b) The detection limit shall be initially determined for the compounds of interest in each test method in a matrix in which there are not target analytes nor interferences at a concentration that would impact the results or the detection limit must be determined in the matrix of interest (see definition of matrix).~~

- ~~c) Detection limits must be determined each time there is a change in the test method that affects how the test is performed, or when a change in instrumentation occurs that affects the sensitivity of the analysis.~~
- ~~d) All sample processing steps of the analytical method shall be included in the determination of the detection limit.~~
- ~~e) All procedures used must be documented. Documentation must include the matrix type. All supporting data must be retained.~~
- ~~f) The laboratory must have established procedures to relate detection limits with quantitation limits.~~
- ~~g) The test method's quantitation limits must be established and must be above the detection limits.~~

~~D.1.3 Data Reduction~~

~~The procedures for data reduction, such as use of linear regression, shall be documented.~~

~~D.1.4 Quality of Standards and Reagents~~

- ~~a) The source of standards shall comply with 5.5.6.2.2.2.~~
- ~~b) Reagent Quality, Water Quality and Checks:
 - ~~1) Reagents - In methods where the purity of reagents is not specified, analytical reagent grade shall be used. Reagents of lesser purity than those specified by the test method shall not be used. The labels on the container should be checked to verify that the purity of the reagents meets the requirements of the particular test method. Such information shall be documented.~~
 - ~~2) Water - The quality of water sources shall be monitored and documented and shall meet method specified requirements.~~
 - ~~3) The laboratory will verify the concentration of titrants in accordance with written laboratory procedures.~~~~

~~D.1.5 Selectivity~~

- ~~a) Absolute retention time and relative retention time aid in the identification of components in chromatographic analyses and to evaluate the effectiveness of a column to separate constituents. The laboratory shall develop and document acceptance criteria for retention time windows.~~
- ~~b) A confirmation shall be performed to verify the compound identification when positive results are detected on a sample from a location that has not been previously tested by the laboratory. Such confirmations shall be performed on organic tests such as pesticides, herbicides, or acid extractable or when recommended by the analytical test method except when the analysis involves the use of a mass spectrometer. Confirmation is required unless stipulated in writing by the client. All confirmation shall be documented.~~
- ~~c) The laboratory shall document acceptance criteria for mass spectral tuning.~~

~~D.1.6 Constant and Consistent Test Conditions~~

- a) ~~The laboratory shall assure that the test instruments consistently operate within the specifications required of the application for which the equipment is used.~~
- b) ~~Glassware Cleaning - Glassware shall be cleaned to meet the sensitivity of the test method.~~

~~Any cleaning and storage procedures that are not specified by the test method shall be documented in laboratory records and SOPs.~~

D.1 Ongoing Quality Control Requirements For Demonstration Of Measurement System Performance For Chemical Testing

This Appendix describes the minimum quality control (QC) procedures that are needed in order to document the quality of data obtained and to demonstrate that the laboratory was functioning in control. These requirements apply to virtually all types of testing (exceptions are noted). The laboratory shall ensure that the essential requirements in this Appendix are incorporated into their method manuals and/or the Laboratory Quality Assurance Plan. In addition to these minimum requirements, the laboratory shall also perform any additional procedures that are called for in the particular method that is being used. The laboratory shall have procedures for the development of acceptance/rejection criteria for the results of the quality control tests where no method or regulatory criteria exists. These criteria shall be based on the MQCs determined in the initial measurement system evaluation and shall be updated periodically based on the results from the analysis of QC samples.

The minimum quality control requirements are summarized in Table D-1

Table D-1

<u>Evaluation Element</u>	<u>Section</u>	<u>Quality Control</u>
<u>System Cleanliness</u>	<u>D.1.2</u>	<u>Method blank</u>
<u>Calibration</u>	<u>D.1.3</u>	<u>Verification check or another calibration, second source Standard</u>
<u>Analytical System Performance</u>	<u>D.1.4</u>	<u>Laboratory Control Sample (LCS)</u>
<u>Analyst Proficiency</u>	<u>D.1.5</u>	<u>Annual performance verification</u>
<u>Limit of Detection</u>	<u>D.1.6</u>	<u>Spike sample at, or near the LOD (when data are reported to LOD)</u>
<u>Limit of Quantitation</u>	<u>D.1.7</u>	<u>Spike at LOQ</u>
<u>Bias</u>	<u>D.1.8</u>	<u>Matrix spike/matrix spike duplicates *</u>
<u>Precision</u>	<u>D.1.8</u>	<u>Matrix spike/matrix spike duplicates, or replicate samples for each batch*</u>
<u>Selectivity</u>	<u>D.1.9</u>	<u>Confirm analyte identity</u> <u>Confirm that quantitative results do not have a positive bias</u>

* This QC test is not required in all applications.

D.1.1 Introduction

All measurement systems must be evaluated prior to their use on actual samples to determine measurement quality characteristics (MQCs) in representative sample-types (See Appendix C). The ongoing QC requirements established in this Appendix shall also be performed. Also, as part of the laboratory's training program, all laboratory staff involved in the analysis of a sample shall have passed a Demonstration of Analyst Proficiency prior to the analysis of any samples

(see D.1.5 and Appendix G). The Demonstration shall be repeated whenever there is a significant change in instrument type, personnel, matrix or test method.

The results of these ongoing QC sample analyses shall be documented and reported and/or available along with the analytical results.

D.1.2 System Cleanliness

A critical component of analytical quality control is making certain that the species or properties whose level has been measured are not artifacts of the measurement system. Such artifacts can be caused by contamination of the instruments, the reagents, the preparation glassware, etc. Method blanks are used to assess the potential contribution of such contamination to the analytical results.

A method blank is used to assess measurement system cleanliness on each preparation batch for possible contamination during the preparation and processing steps. The method blank shall consist of a sample-type that is representative of the associated samples and is known to be free of the analytes of interest. The method blank shall be processed along with and under the same conditions as the associated samples to include all steps of the analytical procedure as if an actual sample was being analyzed. Any samples associated with (same batch) a contaminated method blank shall be reprocessed for analysis or the results reported with appropriate qualification. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

While the goal is to have no detectable contaminants, each method blank must be critically evaluated as to the nature of the interference and the affect on the analysis of each sample within the batch. For purposes of taking corrective action a method blank is considered contaminated if the concentration of a reported analyte in the blank exceeds the greater of:

- 1) the established LOQ;
- 2) 1/10 of the measured concentration, which is above the LOQ, in any sample in the associated batch; or
- 3) 1/10 of the specified regulatory limit, which is above the LOQ, in any sample in the associated batch

Also, a method blank is considered to be contaminated if detected analytes otherwise affect the sample results as per the test method requirements or client MQOs.

If a method blank is determined to be contaminated, the source must be investigated and measures taken to minimize or eliminate the problem. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the affected samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action shall be documented.

D.1.3 Calibration and Calibration Verification

Each day that analyses are to be performed using a particular instrument, the calibration of the instrument must be verified. See Section 5.5.5.2.2 for details.

D.1.4 Analytical System Performance

During routine use of a test method it is important to ensure that the analytical system is operating as expected (i.e., the performance of the system is in conformance with the expectation

established by the initial measurement system evaluation). To document that the system is meeting expectations (and to identify if any problems are developing) a Laboratory Control Sample (LCS) is analyzed, including all steps of sample preparation and analyses, along with each batch of samples.

The LCS is used to evaluate the performance of the total analytical system, including all preparation and analysis steps. Results of the LCS are compared to established criteria and, if found to be outside of these criteria, indicates that the analytical system is out of control. In addition, trends in the LCS from batch to batch may be used as an early warning indication that problems may be developing and permit corrective action to be taken before the system reaches an out of control state. Any affected samples associated with an out of control LCS shall be reprocessed for re-analysis or the results reported with appropriate qualification.

The LCS shall be analyzed at a minimum of 1 per preparation batch. Exceptions would be for those analytes for which no spiking solutions are available such as total suspended solids, total dissolved solids, total volatile solids, total solids, pH, color, odor, temperature, dissolved oxygen or turbidity. In those instances for which no separate preparation method is used (example: volatiles in water) the batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents.

The LCS is a controlled sample-type, known to be free of analytes of interest, spiked with known and verified concentrations of analytes. Alternatively the LCS may consist of a media containing known and verified concentrations of analytes, such as a Certified Reference Material. All spike concentrations should be within the calibration range of the methods. Ideally, the LCS should contain all reportable analytes. The following shall be used in choosing components for the spike mixtures:

The components to be spiked shall be those that are reported to the client, including any permit specified analytes or client requested analytes. Unless otherwise required by a mandated test method or the client, the laboratory shall prepare the LCS using the following guidelines:

- a) For those components that interfere with an accurate assessment such as spiking simultaneously with technical chlordane, toxaphene and PCBs, the spike should be chosen that represents the chemistries and elution patterns of the components to be reported
- b) For those test methods that have extremely long lists of analytes, a representative number may be chosen using the following criteria. However, the laboratory shall insure that all targeted components are included in the spike mixture over a 2 year period.
 - 1) For methods that include 1-10 targets, spike all components;
 - 2) For methods that include 11-20 targets, spike at least 10 or 80%, whichever is greater;
 - 3) For methods with more than 20 targets, spike at least 16 components.
- c) Spike components should be chosen to represent the chemistries of the components to be reported. For chromatographic methods, the entire elution range should be represented by spike components.

The results of the individual batch LCS are calculated in percent recovery (%R) where:

$$\%R = (\text{Observed Value}/\text{True Value})(100)$$

Each individual analyte LCS recovery is compared to the acceptance criteria defined by the client or required by regulation. Where there are no established criteria, the laboratory should refer to the measurement quality characteristics of the method initially determined as part of the Initial

Method Evaluation and updated with ongoing performance data in order to establish the limits.. For spike results outside MQOs or MQCs, corrective action should be documented or the data reported with appropriate data qualifying codes and explanation to the client.

An LCS that is determined to be within the MQOs effectively establishes that the analytical system is in control and validates system performance for the samples in the associated batch. Samples analyzed along with a LCS determined to be out of control (e.g., an LCS failure) shall be considered suspect and the samples reprocessed and re-analyzed or the data reported with appropriate qualification.

If a large number of analytes are in the LCS, then it becomes statistically likely that a few will be outside the control limits. This does not indicate that the system is out of control, and corrective action may not be necessary. In this situation, upper and lower marginal exceedance (ME) limits can be established to assist with the corrective action. If more analytes exceed the LCS control limits than is allowed, or if any one analyte exceeds the ME limits, then the LCS has failed. This marginal exceedance approach is relevant for methods with long lists of analytes. It will not apply to target analyte lists with fewer than 30 analytes. (Note: These ME limits may be established using the MQO process, or are based on 4 times the standard deviation obtained in the Initial Measurement System Evaluation, as updated with ongoing performance data.)

The number of allowable marginal exceedances is as follows:

TABLE D-2. NUMBER OF MARGINAL EXCEEDANCES IN LABORATORY CONTROL SAMPLES

<u>Number of Analytes in LCS</u>	<u>Allowable Number of Marginal Exceedances</u>
<u>>90</u>	<u>5</u>
<u>71-90</u>	<u>4</u>
<u>51-71</u>	<u>3</u>
<u>31-50</u>	<u>2</u>
<u>11-30</u>	<u>1</u>

Marginal exceedances must be sporadic (i. e., random). If the same analyte exceeds the LCS control limit repeatedly, that is an indication that the problem is systemic and something is wrong with the measurement system. The source of error should be located and the appropriate corrective action taken. Laboratories must monitor the application of the sporadic marginal exceedance allowance to the LCS results to ensure random behavior.

D.1.5 Analyst Proficiency

An important aspect of analytical quality control is to determine and document analyst proficiency (i.e., the competency of the analytical team to perform the specific tests that are being conducted). In addition to documentation of education and training, a critical quality control measure that shall also be conducted is periodic demonstration of proficiency (see Section 5.5.2.6.c(3)).

Analyst proficiency shall be demonstrated at least annually for each test method that the analyst/work cell is performing and shall be documented using the form in Appendix G.

D.1.6 Limit of Detection

When the client or regulation requires that quantitative data at the LOD of the measurement system be reported, the laboratory must document that the measurement system is achieving

that detection limit. The demonstration is performed by the analysis of a QC sample periodically (at least quarterly) containing the analyte of concern at no greater than 2-3X the detection limit for single component tests and 1-4 X the detection limits for multi-analyte components. This demonstration shall be conducted for each sample type and for each analyte of concern but need only be used when data at the LOD is to be reported quantitatively. The requirement for ongoing demonstration of LOD does not apply in cases where results reported below LOQ are appropriately qualified.

D.1.7 Limit of Quantitation

When the client or regulation requires that the presence or absence of an analyte at the LOQ of the measurement system be reported, then the laboratory must conduct the necessary quality control procedures to document that the measurement system is achieving the particular LOQ that is being reported. The procedure must include all isolation/concentration steps, and sufficient analyte concentration must be used to ensure a quantitative analyte determination. Attainment of the LOQ (the LOQ is determined using the procedures described in Appendix C) shall be verified by analysis of QC sample containing the analyte of concern at the LOQ. The percent recovery for each analyte shall be determined. The minimum frequency for ongoing evaluation of the LOQ is annually or when a new and unusual matrix is encountered.

Results are compared to the MQOs as published in mandated test methods or provided by the client, or as established in the Initial Measurement System Evaluation and updated with ongoing performance data as appropriate.

D.1.8 Matrix-Specific Bias and Precision

An integral part of determining the quality of laboratory data is documenting that the measurement system is yielding data suitable for the intended purpose (i.e., the bias and precision of the analytical system meets the client MQOs). To demonstrate that the bias and precision of the measurement system meets the MQOs, a matrix spike/matrix spike duplicate (MS/MSD) pair is analyzed.

The frequency of the analysis of MS/MSD may be determined as part of a systematic planning process (e.g. Data Quality Objectives or MQOs) or as specified by a mandated test method. It will therefore be necessary for the laboratory to communicate with clients to determine the clients' needs, to determine which samples constitute a similar matrix type, and to ensure that sufficient sample volume is collected to determine the uncertainty of associated measurement results on samples using the procedures described below. If neither the client nor the test method requires this activity, the procedure described below need not be performed.

The components to be spiked shall be as specified by the mandated test method, by applicable regulation, or by the client. However, all analytes for which quantitative results are to be reported shall be determined. In the event the list of analytes to be determined contain components whose simultaneous presence will interfere with making an accurate assessment (but which are not expected to actually be present simultaneously in actual samples), such as spiking simultaneously with technical chlordane, toxaphene and PCBs, the spike should be chosen that represents the chemistries and elution patterns of the components to be reported.

For those test methods that have extremely long lists of analytes, a representative number may be chosen, using the following criteria for choosing the number of analytes to be spiked. However, the laboratory shall insure that all targeted components are included in the spike mixture over a 2 year period.

- a) For methods that include 1-10 targets, spike all components;
- b) For methods that include 11-20 targets, spike at least 10 or 80%, whichever is greater;

- c) For methods with more than 20 targets, spike at least 16 components. Spike components should be chosen to represent the chemistries of the components to be reported.

For chromatographic methods, the entire elution range should be represented by spike components.

The results from MS/MSD are primarily designed to assess the precision and accuracy of analytical results in a given matrix and are expressed as percent recovery (%R) and relative percent difference (RPD), where:

$$\text{Average \%R} = \frac{[(C (\text{matrix spike}) - C (\text{unspiked sample})) + (C (\text{matrix spike duplicate}) - C (\text{unspiked sample}))] / (2)(\text{Amount of Spike}) \times 100}$$

and

$$\text{Average \%RPD} = \frac{[(\%R (\text{MS}) + \%R (\text{MSD})) / 2] \times 100\%}{}$$

The RPD may be calculated using either the analyte concentration or the percent recovery. Although both approaches are used in practice, use of recoveries to calculate the RPD may result in a different value from that when concentrations are used.

Results are compared to established criteria. Where there are no established criteria, either in the form of MQOs from the client or in mandated methods, the laboratory should refer to the measurement quality characteristics determined as part of the Initial Measurement System Evaluation (see App. C) in order to establish the limits. For results outside MQOs or MQCs, corrective action should be documented or the data reported with appropriate qualification and explanation(s) to the client.

There are several alternatives to using the traditional MS/MSD approach. These include: use of surrogate spikes to measure bias and precision and analysis of replicate samples of the same material to demonstrate acceptable precision. Surrogates are materials that have similar analytical properties to the analytes of concern but which are not naturally found in environmental samples. Surrogates are used most often in organic chromatography test methods and are chosen to reflect the chemistries of the targeted components of the method. Added prior to sample preparation/extraction, they provide a measure of recovery for each individual sample matrix.

When the surrogate approach is employed, the recovery and precision of the surrogates is used as the measure of bias and precision as described above for the MS/MSD approach.

D.1.9 Selectivity

The minimum requirement is to ensure that the measurement system is adequately selective. This includes performing the appropriate instrument set-up and performance checks (e.g., ICP inter-element interference checks, MS tune, determination of chromatography retention time windows).

D.2 Toxicity Testing

These standards apply to laboratories measuring the toxicity and/or bioaccumulation of contaminants in general. They are applicable to toxicity or bioaccumulation test methods for evaluating. They are applicable to toxicity or bioaccumulation test methods for evaluating in effluents (whole effluent toxicity or WET), receiving waters, sediments, elutriates, leachates and soils. In addition to the essential quality control standards described below, some methods may have additional or other requirements based on factors such as the type of matrix evaluated. Additional information can be found in the following methods manuals (or most recent edition):

~~EPA/600/4-91/002, EPA/600/4-91/003, EPA/600/4-90/027F (WET testing), EPA/600/4-90/031 (general aquatic toxicity testing), EPA/600/R-94/025, EPA/600/R-94/024, EPA/503/S-91/001, EPA/823/B-98/004 (sediments and elutriates), EPA/600/3-88/029, EPA/600/3-89/013, ASTM E 1598-94 and ASTM 1676-97 (soils). Additional information can be found in the following methods manuals (or most recent edition): EPA/600/4-91/002, EPA/600/4-91/003, EPA/600/4-90/027F (WET testing), EPA/600/4-~~

~~90/031 (general aquatic toxicity testing), EPA/600/R-94/025, EPA/600/R-94/024, EPA/503/R-91/001, EPA/823/B-98/004 (sediments and elutriates), EPA/600/3-88/029, EPA/600/3-89/013, ASTM E1598-94 and ASTM 1676-97 (soils).~~

D.2.1 Positive and Negative Controls

a) ~~Positive Control - Reference Toxicants - Reference Toxicants~~ — Reference tests indicate ~~the sensitivity of the test organisms being used and indicate the sensitivity of the test organisms being used and demonstrate a laboratory's ability to obtain consistent results with the test method.~~

1) The laboratory must demonstrate its ability to obtain consistent results with reference toxicants in order to attain accreditation in toxicity testing methods by completing a Demonstration of Capability [DOC] before it performs toxicity tests with effluents or other environmental samples for regulatory compliance purposes~~before it performs toxicity tests with effluents or other environmental samples for regulatory compliance purposes.~~

i) ~~To meet this requirement, the intra-laboratory precision must be determined by performing~~ An initial DOC shall consist of five or more acceptable standard reference toxicant [SRT] tests for each test method and species with different batches of organisms and appropriate negative controls (water, sediment, or soil). Initial DOCs shall be prepared in accordance with the requirements of Appendix C.

ii) ~~An intralaboratory coefficient of variation (%CV) is not established for each test method. However, DOC is established by maintenance of SRT test results~~ ona testing laboratory shall maintain control charts. A laboratory shall record for the control performance and reference toxicant statistical endpoint (such as NOEC or ECp) for each test method on control charts. DOC is established where the tests results required in D.2.1a)1)i) above fall within the control limits established in accordance with D.2.1.a)1)iii) below and shall evaluate the intralaboratory variability with a specific reference toxicant for each test method.

iii) For endpoints that are point estimates (ICp, ECp) control charts are constructed by plotting the cumulative mean and the control limits limits which consist of the upper and lower 95% confidence limits (+/- 2 std. dev.). For endpoints from hypothesis tests (NOEC, NOAEC) the values are plotted directly and the control limits consist of one concentration interval above and below the concentration representing central tendency (i.e. the mode).

2) Ongoing laboratory performance shall be demonstrated by performing ~~regularregular-Routine SRT reference toxicant tests reference toxicant tests testing~~ for each test method and species in accordance with the minimum frequency requirements specified in D.2.1.a.3.

i. Intralaboratory precision is determined on an ongoing basis through the use of control charts as established in D.2.1a)1)i) above~~must be determined~~

~~through the use of reference toxicant tests and plotted in quality control charts. must be determined through the use of reference toxicant tests and plotted in quality control charts.~~ The control charts shall be plotted as point estimate values, such as EC25 for chronic tests and LC50 for acute tests, or as appropriate hypothesis test values, such as the NOEC or NOAEC, over time within a laboratory.

~~ii. For endpoints that are point estimates (IC_p, EC_p) control charts are constructed by plotting the cumulative mean and the control limits which consist of the upper and lower 95% confidence limits (+/- 2 std. dev.). For endpoints from hypothesis tests (NOEC, NOAEC) the values are plotted directly and the control limits consist of one concentration interval above and below the concentration representing central tendency (i.e. the mode).~~

~~ii. After initial laboratory DOC is determined, the control limits for an individual test method and species shall be adjusted as additional test results are obtained. After 20 data points are collected for a test method and species, the control chart is maintained using only the last 20 data points, i.e. each successive mean value and control limit is calculated using only the last 20 values.~~

3) The frequency of ongoing laboratory reference toxicant testing shall be as follows: comply with the EPA or state permitting authority requirements. ~~The following minimum frequency shall be met: comply with the EPA or state permitting authority requirements. The following minimum frequency shall be met:~~

~~i. Each batch of test organisms obtained from an outside source, field collection or from laboratory spawning of field-collected species not amenable to routine laboratory culture (for example, sea urchins and bivalve mollusks) must be evaluated with a reference toxicant test of the same type as the environmental toxicity test within the seven days preceding the test or concurrently with the test.~~

~~ii. Test organisms obtained from in-house laboratory cultures must be tested with reference toxicant tests at least once each month for each test method. However, if a given species produced by in-house cultures is used only monthly, or less frequently, a reference toxicant test of the same type must be performed with each environmental toxicity test.~~

~~iii. For test methods and species commonly used in the laboratory, but which are tested on a seasonal basis (e.g. sea urchin fertilization tests), reference toxicant tests must be conducted for each month the method is in use.~~

i. If the laboratory maintains breeding cultures:

1) for test methods conducted at a frequency of greater than quarterly, SRT tests shall be conducted at an ongoing frequency of monthly.

2) for test methods and species commonly used in the laboratory, but which are tested at a frequency of quarterly or less, including those test organisms which are used on a seasonal basis (e.g. sea urchin fertilization tests), SRT tests shall be conducted for each month the method is in use.

ii. If the laboratory does not maintain breeding cultures:

The sensitivity of each batch of organisms received from a supplier shall be determined via a SRT test within seven days preceding the test or concurrently with the test.

- 6) If several variations of a test method are used by the laboratory (e.g. 48-hour static acute, 48-hour renewal acute, 96-hour renewal acute) the reference test for the method shall be based on the variation with the longest exposure and/or greatest degree of laboratory manipulations.
- b) Negative Control - Control, Brine Control, Control Sediment, Control Soil or Dilution Water -
 - 2) Appropriate additional negative controls shall be included when sample adjustments (for example addition of ~~sodium hydroxide for pH adjustment or sodium hydroxide for pH adjustment or thiosulfate for dechlorination~~) or solvent carriers are used in the test.
 - 3) Test Acceptability Criteria (TAC) - The test acceptability criteria ~~(for example, the whole effluent chronic Ceriodaphnia test, requires 80% or greater survival and an average 15 young per female in the controls)~~ (for example, the whole effluent chronic *Ceriodaphnia* test, requires 80% or greater survival and an average 15 young per female in the controls) as specified in the test method must be achieved for both the reference toxicant and the effluent or environmental sample toxicity test. The criteria shall be calculated and shall meet the method specified requirements for performing toxicity tests.

D.2.4 Test Sensitivity

- a) ~~If the Dunnett's procedure is used, the~~ If the Dunnett's procedure is used, the The statistical minimum significant difference (SMSD) shall be calculated according to the formula specified by the test method and reported with the test results.
- b) ~~Estimate the SMSD for non normal distribution and or heterogeneous variances.~~
- b) Point estimates: (LCp, ICp, or ECp) - Confidence intervals shall be reported as a measure of the precision around the point estimate value, when applicable.
- c) The SMSD shall be calculated and reported for only hypothesis test values, such as the NOEC or NOAEC.

D.2.5 Selection of Appropriate Statistical Analysis Methods

- a) If required, methods of data analysis and endpoints are specified by language in the regulation, permit or the test method.
- b) Dose Response Curves – ~~When required, the~~ When required, the The data shall be plotted in the form of a curve relating the dose of the chemical or concentration of sample to cumulative percentage of test organisms demonstrating a response such as death.

D.2.6 Selection and Use of Reagents and Standards

- (c) Only reagent-grade water collected from distillation or deionization units (~~→ 17 megohm resistivity~~) (~~→ 17 megohm resistivity~~) is used to prepare reagents.

D.2.8 Constant and Consistent Test Conditions

- a) If closed refrigerator-sized incubators are used, culturing and testing of organisms shall be separated to avoid ~~loss of cultures due to loss of cultures due to~~ cross-contamination.

- e) Instruments used for routine support measurements of chemical and physical parameters such as pH, DO, conductivity, salinity, alkalinity, hardness, chlorine, ammonia and weight shall be calibrated, and/or standardized per manufacturer's instructions ~~and Section 5.5.5.2.~~ These are support measurements, only the calibration and verification requirements specified at 5.5.5.2.1 apply. ~~Temperature shall be calibrated per section 5.5.5.2.1 and Section 5.5.5.2.~~ Temperature shall be calibrated per section 5.5.5.2.1. All measurements and calibrations shall be documented.
- f) Test temperature shall be maintained as specified for the test method. Temperature control equipment must be adequate to maintain the required test temperature(s). The average daily temperature of the test solutions must be maintained within 1°C ~~of 1°C of the method specified range.~~ selected test temperature, for the duration of the test. ~~selected test temperature, for the duration of the test.~~ The minimum frequency of measurement shall be once per 24 hour period. The test temperature for continuous-flow toxicity tests shall be recorded and monitored continuously. Where electronic data loggers are used, temperature shall be monitored at a frequency sufficient to capture temporal variations of the environmental control system.
- i) The quality of the food used for testing or culturing must be sufficient to allow satisfactory survival, growth and reproduction of the test species as demonstrated by routine reference toxicant tests and negative control performance. ~~For each new batch of laboratory-prepared or lot of commercial food used by the laboratory, the performance of organisms fed with the new food shall be compared with the performance of organisms fed with a food of known quality. If the food is used for culturing, its suitability is determined using a measure that evaluates the effect of food quality on survival and growth or reproduction of each of the relevant test species. Where applicable, foods used only in chronic toxicity tests are evaluated using the reference toxicant regularly employed in the laboratory QA program and compared with results of previous test(s) using a food of known quality. In the case of algae, rotifers or other cultured foods, which are collected as a continuous batch, the quality is assessed as described above, each time new nutrient stocks are prepared, a new starter culture is employed or when a significant change in culture conditions occurs. The laboratory shall have written procedures for the statistical evaluation of food acceptance.~~
- ~~i)j)~~ Food used to culture organisms. ~~Organisms used in bioaccumulation tests must be analyzed at the start of test (baseline) for the target compounds to be measured in the bioaccumulation tests.~~
- ~~i)m)~~ All organisms in a test must be from the same source. Where commercially available certified seeds are used for soil tests.
- ~~i)p)~~ Light intensity shall be maintained as specified in the methods manuals. Measurements shall be made and recorded on a yearly basis. Photoperiod shall be maintained as specified in the test methods and shall be documented at least quarterly at least quarterly annually. ~~For algal and plant tests, the light intensity shall be measured and recorded at the start of each test.~~
- q) ~~At a minimum, during aquatic chronic testing DO and pH shall be measured daily in at least one replicate of each concentration. In static-renewal tests DO must be measured at both the beginning and end of each 24-h exposure period and may be measured in old and new solutions prior to organism transfer, or after organism transfer; pH is measured at the end of each exposure period (i.e. in old solutions).~~
- ~~i)s)~~ The maximum holding time of effluents (elapsed time from sample collection to first use in a test) shall not exceed 36 hours; samples may be used for renewal up to 48 hours after the first use and the last use of the sample in test renewals shall not exceed 72 hours without the permission of the permitting authority and the last use of the sample in

~~test renewals shall not exceed 72 hours without the permission of the permitting authority.~~

~~i)t) All samples shall be chilled to 4°C during or immediately after starting at the time of collection (see requirements in section 5.5.8.3.1).~~

~~i)u) Organisms used in a given test obtained from an outside source must be from the same batch. Chronic tests shall have a minimum of four replicates per treatment.~~

~~v) Chronic tests shall have a minimum of four replicates per treatment.~~

~~x) The culturing of C. dubia shall be adequate such that the blocking parentage can be established.~~

~~z)aa) An individual test may be conditionally acceptable if temperature, dissolved oxygen, pH and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests (see test conditions and test acceptability criteria specified for each test method). The acceptability of the test shall depend on the experience and professional judgment of the technical employe-director and the permitting authority.~~

D.3 Microbiology Testing

D.3.8 Constant and Consistent Test Conditions

- b) Laboratory Equipment
 - 3) Volumetric Equipment

Volumetric equipment shall be ~~calibrated~~verified for accuracy as follows:

- i) equipment with movable parts such as automatic dispensers, dispensers/diluters, and mechanical hand pipettes shall be ~~calibrated~~verified for accuracy quarterly.
- ii) equipment such as filter funnels, bottles, non-class A glassware, and other marked containers shall be ~~calibrated~~calibrated-verified once per lot prior to first use.

D.4 Radiochemical Testing

D.4.1 Negative and Positive Controls

- b) Positive Controls
 - 2) Matrix Spike - Shall be performed at a frequency of one per preparation batch for those methods which do not utilize an internal standard or carrier, for which there is a chemical separation process, and where there is sufficient sample to do so. ~~The exceptions are This includes aqueous samples for gross alpha, gross beta and tritium measurements which shall require matrix spikes for aqueous samples.~~ The results of this analysis shall be
 - 7) Where gamma spectrometry is used to identify and quantitate more than one analyte isotope the laboratory control sample and matrix spike shall contain isotopes that represent the low (e.g. americium-241), medium (e.g. cesium-137) and high (e.g. cobalt-60) energy range of the analyzed gamma spectra. As indicated by these examples the isotopes need not exactly bracket the ~~calibrated~~required energy range or the range over which isotopes are identified and quantitated.

D.4.4 Radiation Measurement System Calibration

Because of the stability and response nature of modern radiation measurement instrumentation, it is not typically necessary to ~~verify~~ calibrate ~~of~~ these systems each day of use. However, verification of calibration is required as outlined in D.4.4 b) below. This section addresses those practices that are necessary for proper calibration and those requirements of section 5.5.5.2.2 (Instrument Calibrations) that are not applicable to some types of radiation measurement instrumentation.

a) Initial Instrument Calibration

- 1) Given that activity detection efficiency is independent of sample activity at all but extreme activity levels, the requirements of subsections f, h and i of 5.5.5.2.2.1 are not applicable to radiochemical method calibrations except mass attenuation in gas-proportional counting and sample quench in liquid scintillation counting. Radiochemistry analytical instruments are subject to calibration when purchased, when the instrument is serviced, when the instrument is moved and when the instrument setting(s) have been changed; and when control charts indicate an out of control condition.

D.4.6 Data Reduction

- c) Minimum Detectable Activity (MDA) – each analytical result shall be reported along with the computed MDA for that specific analysis.

D.5 Air Testing

D.5.3 Method Evaluation

In order to ensure the accuracy of the reported result, the following procedures shall be in place:

- a) Demonstration of Capability – (Sections 5.5.2.6 and 5.5.4.2.2) shall be performed prior to the analysis of any samples and with a ~~significant~~ change in instrument type, personnel, matrix, or test method.

D.5.4 Detection Limits

- c) Detection limits must be determined each time there is a ~~significant~~ change in the test method or instrument type.